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# EVALUATION OF MEMBRANE SYSTEMS FOR WASHING/DEGLYCEROLIZING PACKED RED BLOOD CELLS

Annual Report to
U.S. Army Medical Research & Development
Contract No. DAMD-17-86-C-6142
March 1987

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ANNUAL REPORT

K.R. Pearson
J.M. Radovich
R.J. Wedel

March 20, 1987

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6142

Bend Research, Inc.
64550 Research Road
Bend, Oregon 97701-8599

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This is the annual report from Bend Research, Inc. for the contract entitled "Evaluation of Membrane Systems for Washing/Deglycerolizing Packed Red Blood Cells." The objective of this contract is to develop a membrane-based process for deglycerolizing previously frozen, packed red blood cells (RBCs) prior to transfusion. This membrane-based process should reduce the glycerol concentration in the RBC solution to less than 400 mOsm/kg $H_2O$ using about 2000 ml of wash solution in 35 to 45 minutes. The plasma hemoglobin (Hb) concentration in the washed RBCs should be less than 150 mg/dl. These are the performance standards of centrifugal deglycerolization process.

During the first year of the contract, we successfully demonstrated the feasibility of using commercially available membrane devices (hemodialyzers,

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hemofilters, and plasmapheresis devices) to deglycerolize packed RBCs. Some of the devices removed the glycerol in less than 30 minutes. The membrane devices removed the glycerol more efficiently from previously frozen RBCs than from the glycerol-equilibrated packed RBCs that are routinely used in our studies. At least five units of packed RBCs can be effectively deglycerolized in a single membrane device.

As expected, the flux and glycerol-removal rate can be increased by increasing the transmembrane pressure and blood-flow rate in the membrane device. During parametric studies, we found that the deglycerolization process is not limited by the membrane. We also identified the roller pump in our test loop as a major cause of hemolysis during membrane-based deglycerolization.

## SUMMARY

This is the annual report from Bend Research, Inc., to the U.S. Army Medical Research and Development command for Contract No. DAMD17-86-C-6142, entitled "Evaluation of Membrane Systems for Washing/Deglycerolizing Packed Red Blood Cells." It covers the period from February 10, 1986 to February 9, 1987.

The objective of this contract is to develop a membrane-based process for deglycerolizing previously frozen, packed red blood cells (RBCs) prior to transfusion. To accomplish the objective, our research is focused on 1) testing the feasibility of using various membrane devices for deglycerolization; 2) optimizing the operating conditions of the membrane-based deglycerolization process using glycerol-equilibrated packed RBCs; 3) determining the effectiveness of the deglycerolization process at the optimum operating conditions using previously frozen, packed RBCs; and 4) developing a procedure for reusing the membrane device. The membrane devices should reduce the glycerol concentration in the RBC solution to less than 400 mOsm/kg $H_2O$ using ~2000 ml of wash solution in 35 to 45 minutes. The plasma hemoglobin (Hb) concentration in the washed RBCs should be less than 150 mg/dl.

During the first year of the contract, we successfully demonstrated the feasibility of using commercially available membrane devices (hemodialyzers, hemofilters, and plasmapheresis devices) to deglycerolize packed RBCs. In Task 1: Feasibility Studies, we tested fifteen membrane devices and found that ten of them removed enough glycerol to reduce its concentration in the packed RBCs to less than 400 mOsm/kg $H_2O$; three devices did this in less than 45 minutes, and one device used less than 2000 ml of wash solution. Our studies showed that permeate flux* was the best indicator of deglycerolization performance, because we were testing devices that had different areas of membrane surface and were using packed RBCs with different initial concentrations of glycerol. Our tests also indicated that the roller pump in the test loop, rather than deglycerolization in the membrane device, was the cause of hemolysis during the procedure. A commercially available peristaltic blood pump will be acquired for the studies remaining in the second year of the contract. As a result of the feasibility studies, we selected seven membrane devices for the optimization studies in Task 2.

The results of Task 2: Optimization Studies, showed that the flux could be increased by increasing the transmembrane pressure (TMP) or blood-flow rate in the membrane device. These fluxes were high enough to deglycerolize the RBCs in less than 30 minutes (some in as little as 15 minutes). However, an

*Flux is the volume of solution that passes through the membrane per unit time and unit membrane area. Flux in this case has units of ml/min-$m^2$.

increase in flux did not always result in a proportional decrease in the time required to remove the glycerol. Also, the volume of wash solution required to remove the glycerol was independent of the flux. These results indicated that some factor other than the membrane flux is limiting the deglycerolization process. Whether this factor is related to the method of adding the wash solution to the packed RBCs will be determined as part of the optimization studies that will be completed during the second year of the contract. Based on the results of the optimization studies that were completed this past year, we selected three membrane devices to use in the remaining studies.

As part of the work in Task 3: Effectiveness Studies, we demonstrated that the membrane-based deglycerolization of glycerol-equilibrated, packed RBCs was a conservative estimate of the performance obtained when using previously frozen packed RBCs. Initial studies in Task 4: Development of Reuse Procedures, indicated that a single membrane device can deglycerolize at least five units of packed RBCs without significant deterioration in its performance. These tasks will be completed during the second year of the contract.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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## I. STATEMENT OF THE PROBLEM

The optimal use of whole blood involves its separation into various components, each with specific clinical indications for use. Extensive technology for separating and preserving blood products exists. However, sufficient quantities of specific blood products for the treatment of combat casualties are not always on hand. Extending the shelf life of blood products is one approach to solving this problem of blood product availability.

Red blood cells (RBCs) in concentrated form are one blood product commonly needed in large quantities for the treatment of combat casualties. Stockpiling frozen, packed RBCs that have a shelf life of up to 3 years is one way of deploying that blood product for emergencies. However, employing the frozen RBCs requires a relatively complicated preparation procedure. The frozen RBCs must be thawed and then washed to remove the cryopreservative glycerol before transfusion. The current method for deglycerolizing RBCs relies upon automated or semi-automated centrifuges. These centrifuges are bulky, expensive, sophisticated pieces of machinery that require a high level of user experience and skill for operation. They can be operated only in a limited range of conditions (e.g., power, refrigeration, temperature, cleanliness), and they require a sophisticated maintenance infrastructure. These characteristics are disadvantages when employing the centrifuges in the field to deglycerolize packed RBCs for combat casualties. Other technologies should therefore be examined for their potential to provide a more efficient and simpler method of accomplishing the same mission.

The objective of this contract is to develop a membrane-based process for deglycerolizing previously frozen, packed RBCs by washing them with saline solution. The membrane process sketched in Figure 1 would be an alternative to existing centrifugal deglycerolization methods.

## II. BACKGROUND AND LITERATURE REVIEW

Membrane processes are currently used for many clinical applications that involve the processing of blood or blood products. Membrane devices are commercially available for hemodialysis and hemofiltration, plasmapheresis, and the separation, purification, and concentration of blood plasma components. In each of these applications, the membranes retain the cellular components of the blood but remove water and dissolved solutes--the same separation that must be accomplished during deglycerolization. In membrane-based deglycerolization of packed RBCs, glycerol and saline solution pass through the membrane, while the RBCs are retained by the membrane.

Only a few attempts to deglycerolize packed RBCs using a membrane device have been reported in the literature. Zelman et al. used a dialysis membrane to remove the glycerol from the packed RBCs.[1,2] In their system, the packed RBCs were in a sterile membrane bag, and a dialysis solution circulated outside the bag. The glycerol was removed, but not as efficiently as it is by centrifugal processes. Kleinstrauer et al.[3] developed a mathematical model to describe this dialysis deglycerolization process. Zelman et al. also reported that electroosmosis can be used with dialysis to augment the removal of glycerol.[4,5] However, this process was still not as efficient as are centrifugal processes. The membranes used by Zelman et al. for their deglycerolization studies were dialysis membranes. These membranes are nonporous. Transport of solutes across the membrane is effected by a concentration driving force.

Nonporous membranes are used in commercially available hemodialysis devices. J.M. Radovich, the principal investigator on this research contract, previously conducted preliminary deglycerolization experiments with commercially available hemodialysis membrane devices.[6] Previously frozen packed RBCs were deglycerolized by washing the with saline solution, but the procedure took three to five times longer than centrifugal procedures take.

van Reis et al. used a microporous membrane to deglycerolize packed RBCs that had been equilibrated with glycerol (7). They used a cross-flow membrane test cell that contained 400 $cm^2$ of membrane area. Glycerol is removed from the blood by convective transport (bulk flow) through the micropores of the membrane. Their preliminary results showed that microporous membranes could be used to remove the glycerol. Microporous membranes are used in commercially available plasmapheresis devices.

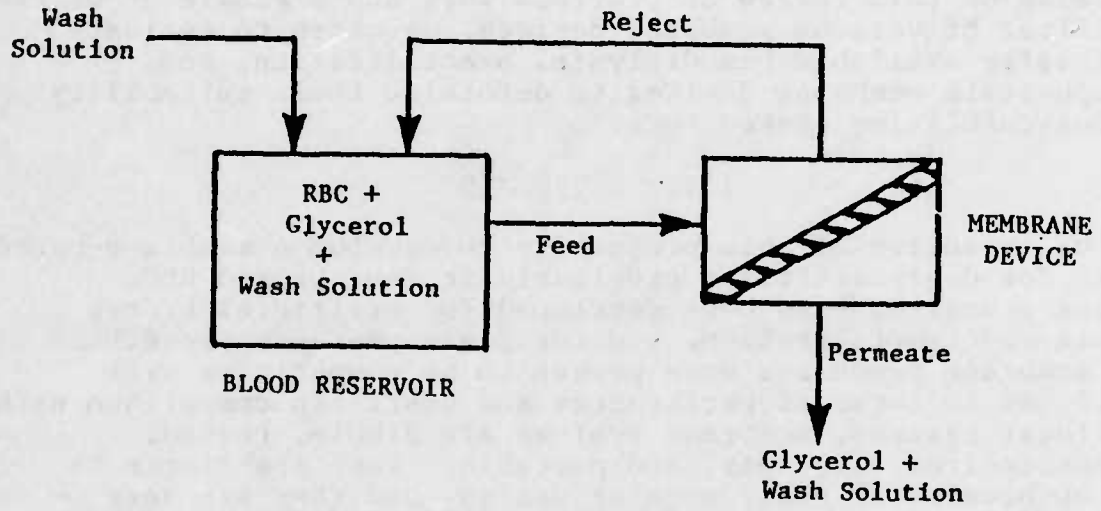


Figure 1. Simplified Diagram of Membrane-Based Deglycerolization Process

Legend: This figure shows the basic components of a membrane-based process for deglycerolizing packed RBCs. A solution of packed RBCs ("blood") circulates from the blood reservoir to the membrane device. The glycerol in the wash solution passes through the membrane wall. The blood, which now contains very little glycerol, is recycled to the blood reservoir where more wash solution is added. The wash solution (normal saline) extracts the glycerol from the RBCs.

Other applications of interest, but not directly applicable to our research, have also been reported. Hemodialysis-type membranes were used to concentrate blood by removing excess plasma water for treatment of hemodilution.[8-10] A flat-sheet membrane system was used to prepare human erythrocyte "ghost" membranes from hemolysate solutions.[11] Exploratory work with hollow-fiber membranes on washing outdated, packed RBCs in preparation for lysing has been carried out as part of the research program on preparing hemoglobin solutions at Letterman Army Institute of Research, Blood Research Divison.[12]

Based on this review of previous work and a knowledge of the capabilites of various membrane devices, we chose to evaluate commercially available hemodialysis, hemofiltration, and plasmapheresis membrane devices to determine their suitability for deglycerolizing packed RBCs.

## III. RATIONALE

The objective of this project is to develop a membrane-based process for deglycerolizing previously frozen, packed RBC. Membrane processes have been developed for artificial kidney dialysis and hemofiltration, and for plasmapheresis procedures. These membrane processes have proven to be competitive with centrifuges in terms of performance and cost. In comparison with centrifugal systems, membrane systems are simple, rugged, maintenance-free, low-cost, and portable. They are simple to scale up because of their modular design, and they are very flexible in meeting changing requirements and are thus capable of operating in a wide range of environments.

The adaptability and ease of use inherent in membrane systems is well illustrated by the increase in home dialysis treatment. These systems also can be readily adapted to sterile docking procedures, as demonstrated by the technology for home dialysis and continuous ambulatory peritoneal dialysis. The simplicity and compactness of membrane systems make them particularly attractive for military applications in the field, such as deployment in the blood-banking unit of an International Standard Organization shelter. Completion of this program will determine whether membrane systems can compete with centrifugal-based procedures for deglycerolizing previously frozen RBCs.

Our research is focused on 1) testing the feasibility of using various hemodialysis, hemofiltration, and plasmapheresis membrane devices for deglycerolization; 2) optimizing the operating conditions (transmembrane pressure [TMP], blood-flow rate, wash-solution addition rate) of the membrane-based deglycerolization process using glycerol-equilibrated packed RBCs; 3) determining the effectiveness (in terms of the percent recovery of hemoglobin [Hb]) of the deglycerolization process at the optimum operating condition using previously frozen, packed RBCs; and 4) developing a procedure for reusing the membrane devices.

The membrane devices should reduce the glycerol concentration in the RBC solution to less than 400 mOsm/kg $H_2O$ in 35 to 45 minutes using approximately 2000 ml of wash solution. The concentration of free Hb (plasma Hb) in the deglycerolized RBC solution should be less than 150 mg/dl. These standards were developed for RBCs that have been deglycerolized by centrifugal procedures.

## IV. EXPERIMENTAL APPARATUS AND METHODS

### IV.A. DEGLYCEROLIZATION TEST SYSTEM

We constructed a test system for evaluating the deglycerolization performance of the various membrane devices. A simplified flow diagram of the test loop is shown in Figure 2, and a list of its major components is given in Table I. The packed-RBC solution ("blood") is pumped from the 2-L Erlenmeyer flask reservoir by a variable-speed roller pump. The blood passes through a standard blood filter before it flows through the fiber lumen of the membrane device. The blood returns to the reservoir after passing through another blood filter.

The blood-flow rate is controlled by adjusting the pump speed. The TMP in the membrane device is controlled by adjusting the pinch-clamp valve on the outlet tubing. The pressures of the inlet blood stream and outlet blood stream of the membrane device are measured by in-line pressure transducers and displayed on digital-readout meters. The tubing connections between the saline wash-solution reservoir and the blood reservoir allow the saline wash solution to be added to the blood reservoir at the same rate as that at which the permeate is withdrawn through the membrane device. The added wash solution and the blood are continuously mixed by agitating the blood reservoir using a shaker platform.

The permeate, which consists of saline solution, glycerol, and proteins that pass through the membrane, is collected from the region outside the hollow fibers. The volume of permeate collected in a graduated cylinder during a given time is used to calculate the permeate flux* by dividing this volume by the membrane surface area.

### IV.B. OPERATING PROCEDURE

The protocol used to deglycerolize packed RBC in the membrane devices is shown in Figure 3. Units of packed RBCs (approximately 250 ml) are obtained from the American Red Cross

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*Flux is the volume of the solution that passes through the membrane per unit time and per unit membrane area. Flux in this case has units of $ml/min\text{-}m^2$.

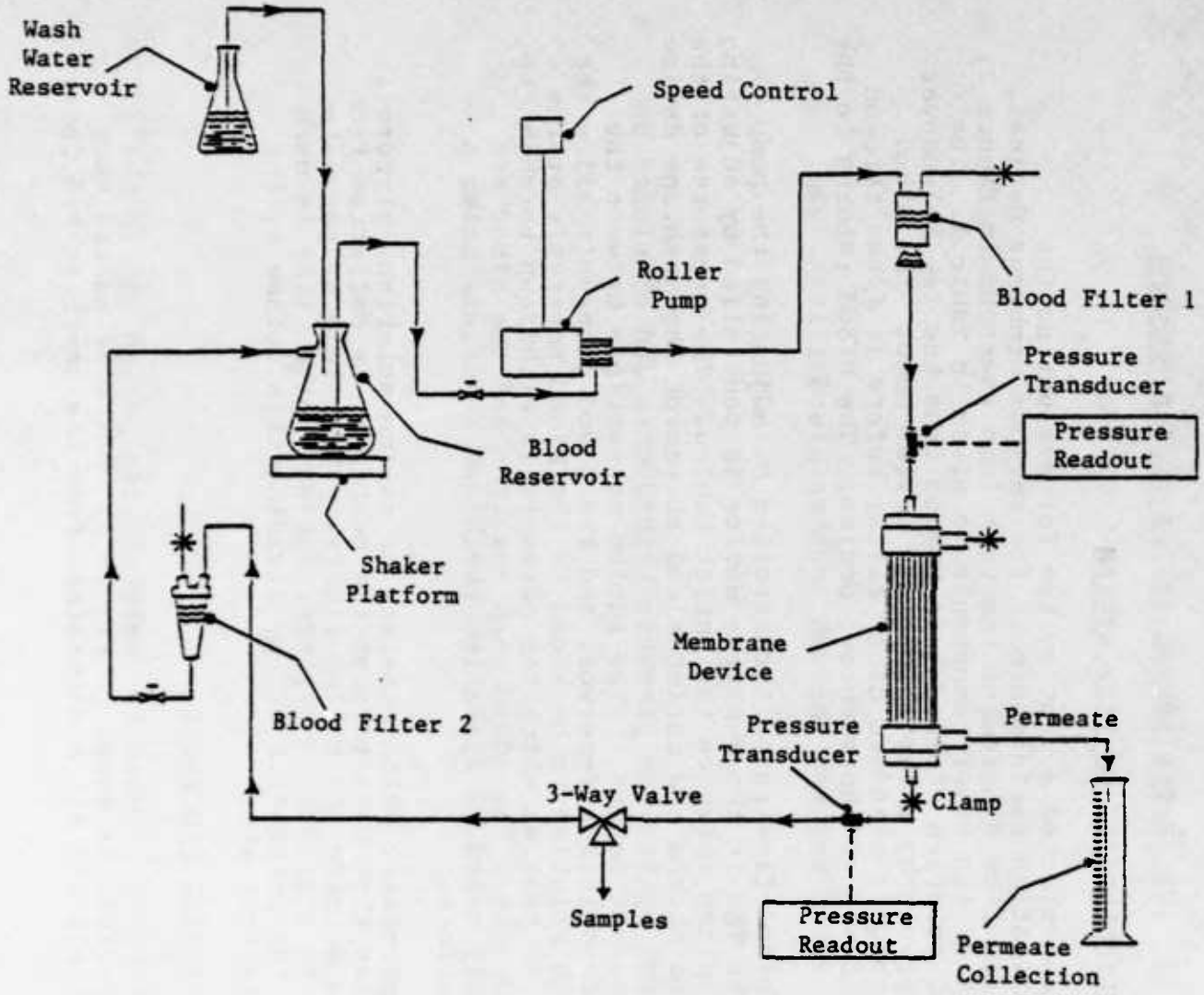


Figure 2. Flow Diagram of Test Loop for Membrane-Based Deglycerolization of Packed RBC

Legend: This figure is a simplified process-flow diagram of the test loop used in the membrane-based deglycerolization process. The blood is pumped from the reservoir through a filter and into the fiber lumen of the membrane device. Glycerol and some wash solution pass through the walls of the fibers. The packed RBCs return to the reservoir, where additional wash solution is added to maintain a constant volume in the reservoir.

| Component | Model No. | Manufacturer and Location |
|---|---|---|
| Shaker platform | R4140 | American DADE, Miami, FL |
| Roller pump and speed controller | 7553-20<br>357778 | Cole Palmer, Chicago, IL<br>Barnant Co., Div. of Cole Palmer, Barrington, IL |
| Blood filter 1 | U006551 | Fenwal, Deerfield, IL |
| Blood filter 2 | B86-004-39 | LifeMed, Compton, CA |
| Pressure transducer | 150PC | Honeywell, San Jose, CA |
| Digital pressure readout | DP200052 | Omega Engineering, Stamford, CT |

Table I. Major Components of the Deglycerolization Test Loop

Legend: This table lists the major pieces of equipment that are used in the membrane-based deglycerolization test loop. The model number and the manufacturer's name and location are listed for each piece of equipment.

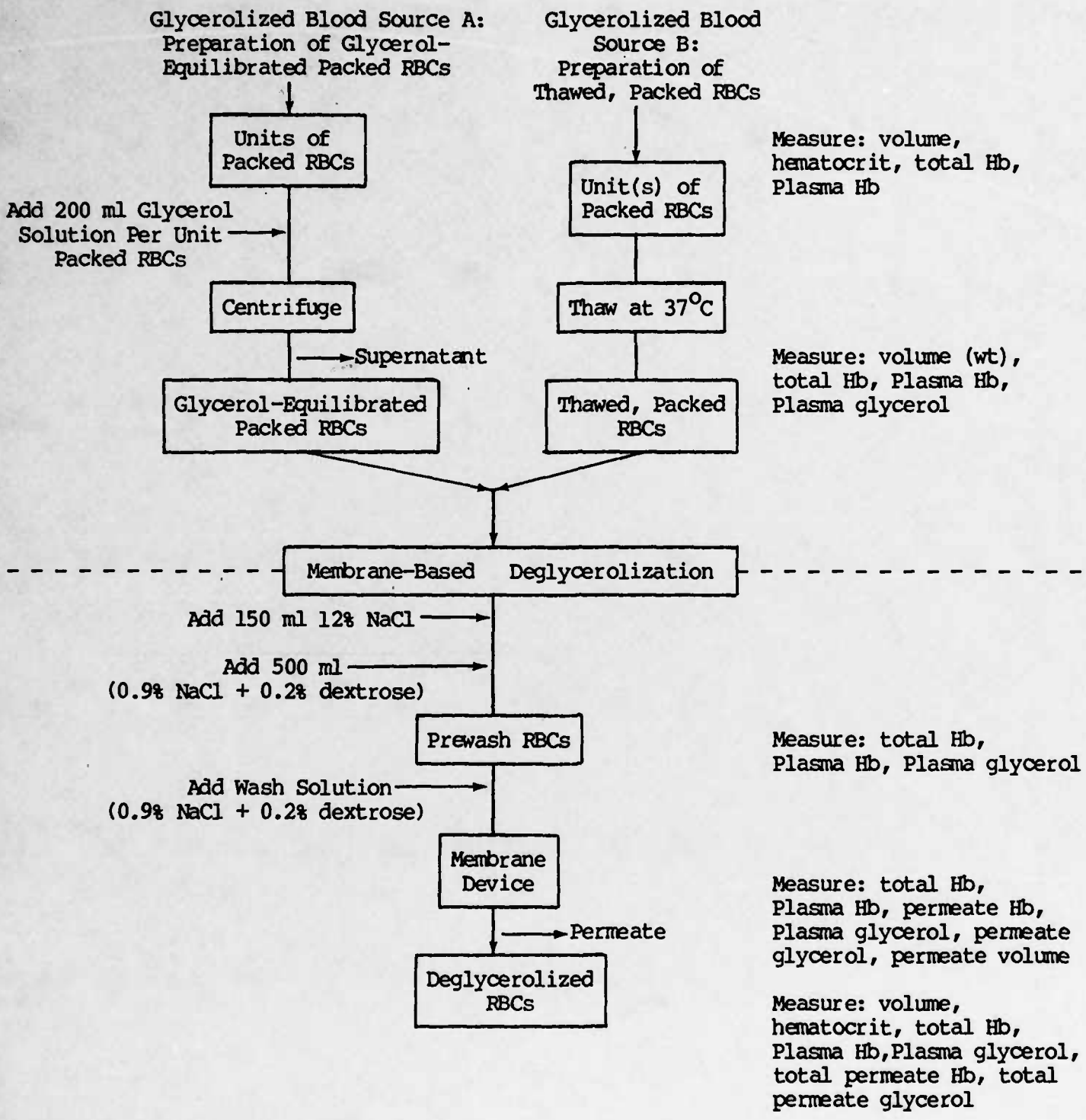


Figure 3. Protocol for Membrane-Based Deglycerolization of Packed RBCs

Legend: This diagram shows the steps for preparing glycerol-equilibrated RBCs or thawed RBCs and for deglycerolizing the RBCs in a membrane device. The analytical measurements that are made during these steps are indicated on the right-hand side of the diagram.

Pacific Northwest Regional Blood Services in Portland, Oregon.** Before the start of each experiment, the volume and hematocrit of the unit and the Hb concentration in the blood and plasma are measured. The unit is then equilibrated with glycerol.[13] A glycerol solution (200 ml of sterile water containing 57.1 g glycerol, 1.6 g sodium lactate, 0.04 g $MgCl_2$, 0.03 g KCl, and 0.08 g disodium phosphate) is added to the packed-RBC unit at a rate of 100 ml/min with continuous mixing. After equilibrating the RBC solution for 15 minutes, it is then centrifuged at 1250 G for 10 minutes. The concentrated RBCs are put in the 2-L Erlenmeyer flask. The Hb concentration in the blood and plasma and the glycerol concentration in the plasma are measured. The RBCs are diluted with 150 ml of 12 wt% NaCl solution. Then, 500 ml of saline wash solution (0.9 wt% NaCl + 0.2 wt% dextrose) is added at the rate of 100 ml/min with continuous mixing. The Hb concentration in the blood and plasma and the glycerol concentration in the plasma of this "prewash" RBC solution are measured.

During the testing of a membrane device, the blood-flow rate and the TMP are kept constant. The addition rate of the saline wash solution is a dependent variable that changes as the permeate rate changes. As the volume of blood in the reservoir decreases due to removal of wash solutions by the membrane device, it is replaced at the same rate by wash solution so that a constant blood volume is maintained. This method of operation is known as diafiltration. Samples of the blood and permeate are taken periodically throughout the test. The Hb and glycerol concentration are measured. At the end of the deglycerolization procedure, the volume of the RBC solution, its hematocrit and total Hb concentration, and the Hb and glycerol concentration in the plasma are measured. The Hb and glycerol concentration in the total collected permeate are also measured.

## IV.C. ANALYTICAL METHODS

The hematocrit of the blood samples is found by centrifuging (Beckman TJ-6 table-top Centrifuge, Palo Alto, California) a 1-ml sample in a graduated, 100-mm Wintrobe tube at 2500 G for 30 minutes. The hematocrit is the ratio of the packed-RBC height to the total fluid height in the tube.

The Hb concentration of the various samples is determined by a modified Drabkins procedure.[14] The spectrophotometric readings to determine the Hb concentration are made with a Perkin-Elmer, UV-VIS 552 Spectrophotometer (Oak Brook, Illinois) at 540 nm and compared with a cyanomethameglobin standard. Drabkins reagent is used as a blank to zero the spectrophotometer.

The glycerol concentration of the samples was determined by measuring the osmolality with an Advanced Instruments Digimatic

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**Hematocrit is the volume percent of RBCs in the blood.

Osmometer, Model 3DII (Needham Heights, MA). Osmolality is determined by measuring the freezing point depression of the sample in the Osmometer. The results are expressed in terms of milliosmoles (mOsm) per kilogram water.

## V. RESULTS AND DISCUSSION

### Task 1. Feasibility Studies

#### A. Deglycerolization Performance

The purpose of these studies was to determine whether commercially available hemodialysis, hemofiltration, or plasmapheresis membrane devices could be used to deglycerolize packed RBCs. At this stage of the project, we were interested primarily in whether the devices could decrease the glycerol content of the packed RBC solution to less than 400 mOsm/kg $H_2O$. The elapsed time, the volume of wash solution required, and the Hb concentration in the deglycerolized RBCs were of secondary importance.

The various membrane devices (and some of their characteristics) that we obtained for our feasibility tests are listed in Table II. The membrane devices selected represent the range of types and characteristics that are commercially available for hemodialysis, hemofiltration, and plasmapheresis. All of them are hollow-fiber membrane cartridges, with the exception of the Gambro Lundia and Biospal, which are flat-sheet devices. These membranes were tested for permeate (saline wash solution and glycerol) flux, and the glycerol and Hb concentration in the washed RBCs were evaluated. The standard operating conditions (which were designed for hemodialysis, hemofiltration, or plasmapheresis) as recommended by the manufacturer were used in these tests.

The performance results for all membrane devices tested during the feasibility studies are summarized in Tables III and IV. As shown in Table III, six of the ten dialyzers and four of the five plasmapheresis devices tested reduced the glycerol concentration of the washed RBCs to less than the required 400 mOsm/kg $H_2O$. Three devices reached that glycerol concentration in less than 45 minutes, but all needed more than 2000 ml of wash solution. One of the membrane devices in the feasibility tests used less than 2000 ml wash solution to achieve the target glycerol concentration but it took 240 minutes. A comparison of the flux data in Table III shows that, in general, the hemofilters and plasmapheresis devices have higher fluxes than do the dialysis devices, and thus would be better suited for deglycerolizing blood. The flux usually remained constant during deglycerolization. As shown in Table IV, five of the membrane devices deglycerolized the packed RBCs such that the final Hb concentration in the plasma was less than the 150-mg/dl standard.

| Membrane Device* | Membrane | Surface Area ($m^2$) | Fiber Characteristics | | Number of Fibers |
|---|---|---|---|---|---|
| | | | Diameter (μm) | Wall Thickness (μm) | |
| Dialysis | | | | | |
| Erika, C-10 | Cuprommonium rayon (Cuprophan) | 1.1 | 200 | 10 | 8,970 |
| CD Medical C-DAK 135 | Saponified cellulose ester | 1.4 | 210 | 25 | 10,800 |
| Travenol ST-15 | Cuprophan | 0.9 | 200 | 8 | 6,500 |
| Toray B1-L | PMMA | 2.0 | 240 | 30 | 14,500 |
| Toray B1-200 | PMMA | 1.6 | 200 | 25 | 12,700 |
| Terumo TAF06 | Regenerated cellulose | 0.6 | 205 | 12 | 5,700 |
| Gambro Lundia 10-5-L | Cuprophan | 1.4 | Flat | 11.5 | Plate |
| Hemoflow F40 | Polysulfone | 0.65 | 200 | 40 | N/A |
| Biospal 2400S | Acrylonitrile/Na methallyl 4A sulfonate copolymer | 1.0 | Flat | 22 | Plate |
| Biospal 1200S | Acrylonitrile/Na methallyl 4A sulfonate copolymer | 0.5 | Flat | 22 | Plate |
| CD Medical C-DAK Duoflux | Cellulose acetate | 1.4 | 220 | 30 | 10,440 |

Table II. Membrane Devices Tested for Deglycerolizing Packed RBCs (continued next page)

| Membrane Device* | Membrane | Surface Area ($m^2$) | Fiber Characteristics | | Number of Fibers |
|---|---|---|---|---|---|
| | | | Diameter (μm) | Wall Thickness (μm) | |
| <u>Hemofiltration</u> | | | | | |
| Amicon D-30 | Polysulfone | 0.55 | N/A | N/A | 5,000 |
| CD Medical Hemoconcentrator | Cellulose acetate | 0.9 | 220 | 30 | 9,000 |
| <u>Plasmapheresis</u> | | | | | |
| Asahi Plasma-separator | Hydrophilized polyethylene | 0.18 | 340 | 50 | N/A |
| Organon Teknika Plasmapur | Polypropylene | 0.07 | 330 | 150 | 400 |
| Organon Teknika Curesis | Polypropylene | 0.12 | 330 | 150 | N/A |
| Toray Plasma Separator | PMMA | 0.5 | 330 | 90 | 2,750 |
| Fresenius Plasmaflux P2 | Polypropylene | 0.5 | 330 | 150 | N/A |

*Manufacturer, brand name or model
N/A = not available

Table II. Membrane Devices Tested for Deglycerolizing Packed RBCs (continued)

Legend: This table lists the characteristics of the membrane devices that were tested during the feasibility studies to determine whether they could remove the glycerol from glycerol-equilibrated packed RBCs. The characteristics of the membrane in each device were obtained from the manufacturer's literature.

| Membrane Device* | Membrane Area ($m^2$) | TMP (mmHg) | Initial Glycerol Concentration (mOsm/kg $H_2O$) | Conditions at Which Glycerol Concentration was 400 mOsm/kg $H_2O$ | | |
|---|---|---|---|---|---|---|
| | | | | Flux ($ml/min-m^2$) | Time Elapsed (min) | Permeate Volume (ml) |
| **Dialysis** | | | | | | |
| Terumo TAFO6** | 1.0 | 170 | 1512 | 4.4 | 228 | 1130 |
| | 1.0 | 170 | 1806 | 4.5 | 290 | 1400 |
| Gambro Lundia 10-5L** | 1.1 | 200 | 2081 | 6.9 | 195 | 1700 |
| | 1.1 | 200 | 1850 | 7.0 | 220 | 2900 |
| Travenol ST-15** | 0.9 | 100 | 2589 | 2.2 | 260 | 500 |
| | 0.9 | 100 | 1731 | 1.9 | 210 | 350 |
| C-DAK 135** | 1.4 | 30 | N/A | 0.55 | 240 | 200 |
| Hemoflow F40 | 0.65 | 190 | 1728 | 127 | 30 | 2800 |
| Toray B1-200 | 1.56 | 200 | 1527 | 19 | 86 | 2950 |
| Erika C-10 | 1.1 | 150 | 1232+ | 6.4 | 240 | 1900 |
| Toray B1-L | 2.0 | 200 | 853+ | 46 | 37 | 2900 |
| | 2.0 | 200 | 966+ | 30 | 39 | 2550 |
| Biospal 2400S | 1.0 | 200 | 1822 | 66 | 32 | 2300 |
| Biospal 1200S | 0.5 | 280 | — | 93 | 59 | 2800 |
| C-DAK Duoflux | 1.4 | 200 | 2620 | 27 | 73 | 3000 |
| **Hemofilters** | | | | | | |
| Amicon D-30 | 0.55 | 120 | 2032 | 91 | 70 | 2700 |
| CD Medical Hemoconcentrator | 0.9 | 190 | 1967 | 23 | 105 | 2400 |

*,**Manufacturer, brand name or model
+Did not reach 400-mOsm point, so reported values are extrapolations
+Initial permeate sample

Table III. Deglycerolization Performance of Membrane Devices
(continued next page)

| Membrane Device* | Membrane Area ($m^2$) | TMP (mmHg) | Initial Glycerol Concentration (mOsm/kg $H_2O$) | Conditions at Which Glycerol Concentration was 400 mOsm/kg $H_2O$ | | |
|---|---|---|---|---|---|---|
| | | | | Flux (ml/min-$m^2$) | Time Elapsed (min) | Permeate Volume (ml) |
| <u>Plasmapheresis</u> | | | | | | |
| Plasmapur | 0.07 | 50 | 2015 | 190 | 220 | 2800 |
| Toray Plasma Separator | 0.5 | 50 | 1787 | 59 | 85 | 2650 |
| Asahi Plasma-separator | 0.18 | 30 | 3224+ | 50 | 240 | 2480 |
| | 0.18 | 30 | 1315+ | 81 | 170 | 2340 |
| Plasmaflux P2 | 0.5 | 50 | 1717 | 37 | 258 | 3100 |
| Curesis | 0.12 | 140 | 2660 | 193 | 115 | 2800 |

*Manufacturer, brand name or model
**Did not reach 400-mOsm point, so reported values are extrapolations
+Initial permeate sample

Table III. Deglycerolization Performance of Membrane Devices (continued)

Legend: This table lists the results of our deglycrolization feasibility studies using different membrane devices. The area of the membrane in the device, the TMP at which the device was operated, and the initial glycerol concentration in the blood before washing are listed. The performance of the device during the deglycerolization process is given in terms of the flux, the elapsed time, and the cummulative volume of permeate collected when the concentration of the glycerol in the blood decreased to 400 mOsm/kg $H_2O$.

| Membrane Device* | Initial Packed-RBC Volume (ml) | Initial Hb Concentration | | Final Washed-RBC Volume (ml) | Final Hb Concentration | |
|---|---|---|---|---|---|---|
| | | Total (g/dl) | Plasma (mg/dl) | | Total (g/dl) | Plasma (mg/dl) |
| Dialysis | | | | | | |
| Terumo TAF06 | 235 | 20.2 | 123 | 930 | 4.61 | 1016 |
| | 240 | 18.8 | 143 | 1120 | 3.60 | 410 |
| Gambro | 230 | 19.4 | 200 | 1350 | 3.16 | 686 |
| Lundia | 235 | 17.7 | 153 | 1420 | 2.21 | 293 |
| Travenol | 250 | 13.2 | Trace | 900 | 4.15 | 537 |
| ST-15 | 220 | 12.9 | 106 | 850 | 4.05 | 670 |
| Erika C-10 | 250 | N/A | N/A | 893 | 3.85 | N/A |
| Toray B1-L | 230 | 18.7 | 140 | 1008 | 4.03 | 226 |
| | 215 | 18.6 | Trace | 990 | 4.00 | 120 |
| Hemoflow F40 | 285 | 24.2 | Trace | 1140 | 9.38 | 297 |
| | 300 | 8.1 | 140 | 895 | 4.62 | 720 |
| Biospal 2400S | 280 | 15.2 | 183 | 3310 | 4.58 | 250 |
| Biospal 1200S | 280 | 18.6 | 99 | 975 | 5.66 | 194 |
| B1 200 | 250 | 5.2 | 0 | 850 | 3.69 | 430 |
| C-DAK Duoflux | 290 | 23.3 | 157 | 2810 | 6.62 | 301 |
| Hemofiltration | | | | | | |
| Amicon D-30 | 300 | 14.2 | Trace | 1150 | 13.8 | 373 |
| CD Medical | 290 | 16.4 | 113 | 910 | 5.15 | 303 |
| Hemoconcentrator | 280 | 12.7 | 373 | 860 | 5.43 | 493 |

*Manufacturer, brand name or model
N/A = not available

Table IV. Hemoglobin Concentrations for Membrane-Based Deglycerolization Test (continued next page)

| Membrane Device* | Initial Packed-RBC Volume (ml) | Initial Hb Concentration | | Final Washed-RBC Volume (ml) | Final Hb Concentration | |
|---|---|---|---|---|---|---|
| | | Total (g/dl) | Plasma (mg/dl) | | Total (g/dl) | Plasma (mg/dl) |
| Plasmapheresis | | | | | | |
| Plasmapur | 265 | 32.5 | 339 | 1025 | 6.00 | 497 |
| Asahi Plasma-separator | 300<br>260 | 19.5<br>16.4 | 698<br>856 | 1000<br>885 | 5.20<br>5.13 | 543<br>353 |
| Toray Plasma Separator | 230 | 13.3 | Trace | 860 | 3.14 | 110 |
| Plasmaflux P2 | 345 | 18.2 | 205 | 860 | 3.91 | 138 |
| Curesis | 280 | 9.1 | 273 | 790 | 3.63 | 80 |

*Manufacturer, brand name or model
N/A = not available

Table IV. Hemoglobin Concentrations for Membrane-Based Deglycerolization Test (continued)

Legend: This table gives the volume and Hb concentration of the packed RBC solutions before and after deglycerolization in the feasibility tests. The total Hb concentration is the concentration of Hb in the RBCs plus the Hb concentration in the solution. The Hb concentration in the plasma is the concentration in the solution outside the RBCs.

B. Flux as a Performance Indicator

Although the time required to decrease the glycerol concentration in the blood to 400 mOsm/kg $H_2O$ is one of the performance standards, it is not the best measure of the deglycerolization performance of the membrane devices, because 1) the devices contain different areas of membrane surface and are thus able to process different volumes of fluid in a given time, and 2) the initial glycerol concentration in the blood varies because the volume of a unit of packed RBCs varies. A comparison of the time-average permeate fluxes is a better basis for evaluating the membrane devices' ability to remove the glycerol and its wash solution from the blood. The flux is

independent of the membrane area and the glycerol concentration in the feed. The higher the flux, the more rapidly glycerol will be removed if the membrane is limiting.

Based on the flux, the membrane area that would be needed to remove 2000 ml of saline wash solution containing glycerol from blood in 30 minutes (4000 ml/hr) was calculated using the following formula:

membrane area = wash-solution removal rate/flux.

The calculated areas are listed in Table V. Membrane devices of the sizes calculated for a 4000 ml/hr deglycerolization rate could be fabricated easily. However, the resulting Erika C-10 device (10.4 $m^2$) would be too large to be practical for this application.

Based on the results of our feasibility studies, we selected five membrane devices for the optimization studies in Task 2. These membranes are the Hemoflow F40, Biospal 1200S, Amicon D-30, Organon Teknika Plasmapur, and the Asahi Plasmaseparator. These membranes were selected because they exhibited the highest permeate fluxes at the standard operating conditions in the feasibility studies, and they have been designed to operate at an even wider range of conditions. We also selected two Toray B1 membranes (B1-200 and B1-L) to determine whether a change in operating conditions would significantly improve the fluxes of these membranes.

C. Predicting the Performance of Hemodialyzers and Hemofilters

Although we evaluated a total of fifteen membrane devices (rather than ten as originally proposed) in the feasibility studies, many more devices might be suitable for deglycerolization. Testing each of these devices would be time-consuming and would take us beyond the scope of this project. Therefore, we tried to correlate deglycerolization performance with some known performance parameter that is characteristic of the commercially available membrane devices. For hemodialyzers and hemofilters, the ultrafiltration rate is such a parameter. This is a measure of the fluid-removal rate by the membrane device during hemodialysis/hemofiltration at a given TMP.

Figure 4 is a plot of the experimental flux (ml/min-$m^2$) of wash solution during deglycerolization versus an ultrafiltration-rate factor (URF) reported by the manufacturer of the membrane device. Each datum point represents the value of a different device. URF is the ultrafiltration rate per unit TMP per unit membrane area (ml/hr-$m^2$-mmHg). It is also known as an "effective permeability." We can use this information to search the manufacturer's literature for hemodialyzers and hemofilters that may have acceptable fluxes for deglycerolization, i.e., that have

| Membrane Device* | Membrane | Flux ($ml/min-m^2$) | Area to Process 4000 ml/hr ($m^2$) | TMP (mmHg) |
|---|---|---|---|---|
| Dialysis | | | | |
| Erika C-10 | Cuprophan | 6.4 | 10.4 | 150 |
| Toray Bl-L | PMMA | 38 | 1.8 | 200 |
| Toray Bl-200 | PMMA | 19 | 3.5 | 200 |
| Hemoflow F40 | Polysulfone | 127 | 0.5 | 190 |
| Biospal 2400 | Acrylonitrile/Na methallyl sulfonate copolymer | 66 | 1.0 | 200 |
| Biospal 1200S | Acrylonitrile/Na methallyl sulfonate copolymer | 93 | 0.7 | 280 |
| C-DAK DuoFlux | Cellulose acetate | 27 | 2.5 | 200 |
| Hemofiltration | | | | |
| Amicon D-30 | Polysulfone | 91 | 0.7 | 120 |
| CD Medical Hemoconcentrator | Cellulose acetate | 25 | 2.7 | 190 |
| Plasmapheresis | | | | |
| Asahi Plasma-separator | Hydrophilyzed polyethylene | 66 | 1.0 | 30 |
| Organon Teknika Plasmapur | Polypropylene | 190 | 0.4 | 50 |

*Manufacturer, brand name or model

Table V. Membrane Performance and Area Required to Process 4000 ml/hr of Wash Solution (continued next page)

| Membrane Device* | Membrane | Flux ($ml/min-m^2$) | Area to Process 4000 ml/hr ($m^2$) | TMP (mmHg) |
|---|---|---|---|---|
| Plasmapheresis | | | | |
| Toray Plasma Separator | PMMA | 59 | 1.1 | 50 |
| Plasmaflux P2 | Polypropylene | 37 | 1.8 | 50 |
| Curesis | Polypropylene | 193 | 0.8 | 140 |
| *Manufacturer, brand name or model | | | | |

Table V. Membrane Performance and Area Required to Process 4000 ml/hr of Wash Solution

Legend: This table presents the calculated areas of each membrane device that would be required to remove 4000 ml/hr of wash solution as permeate at the listed TMP. This removal rate is equivalent to using 2000 ml of wash solution in 30 minutes--the amount of solution typically used by centrifuges. The area was calculated by dividing the 4000 ml/hr by the flux that was determined during the deglycerolization feasibility tests.

URFs of 15 or higher. We have not been able to find a similar performance factor that could be used to screen plasmapheresis membrane devices.

D. Hemolysis Studies

The Hb concentrations in the blood and plasma at various times during the deglycerolization procedure are given in Table IV. The plasma Hb concentrations are the concentrations in the supernatant obtained by centrifuging the blood samples. An Hb concentration in the plasma higher than the allowable limit of 150 mg/dl would indicate excessive lysis of the blood cells and render the deglycerolized RBCs unsuitable for transfusion. As shown in Table IV, the plasma Hb concentration in most of the washed RBC samples was higher than the allowable maximum.

A control experiment was conducted to determine if the high Hb concentration was due to hemolysis during the membrane-based deglycerolization procedure. For this experiment, the membrane device was removed from the test loop. We measured the plasma Hb

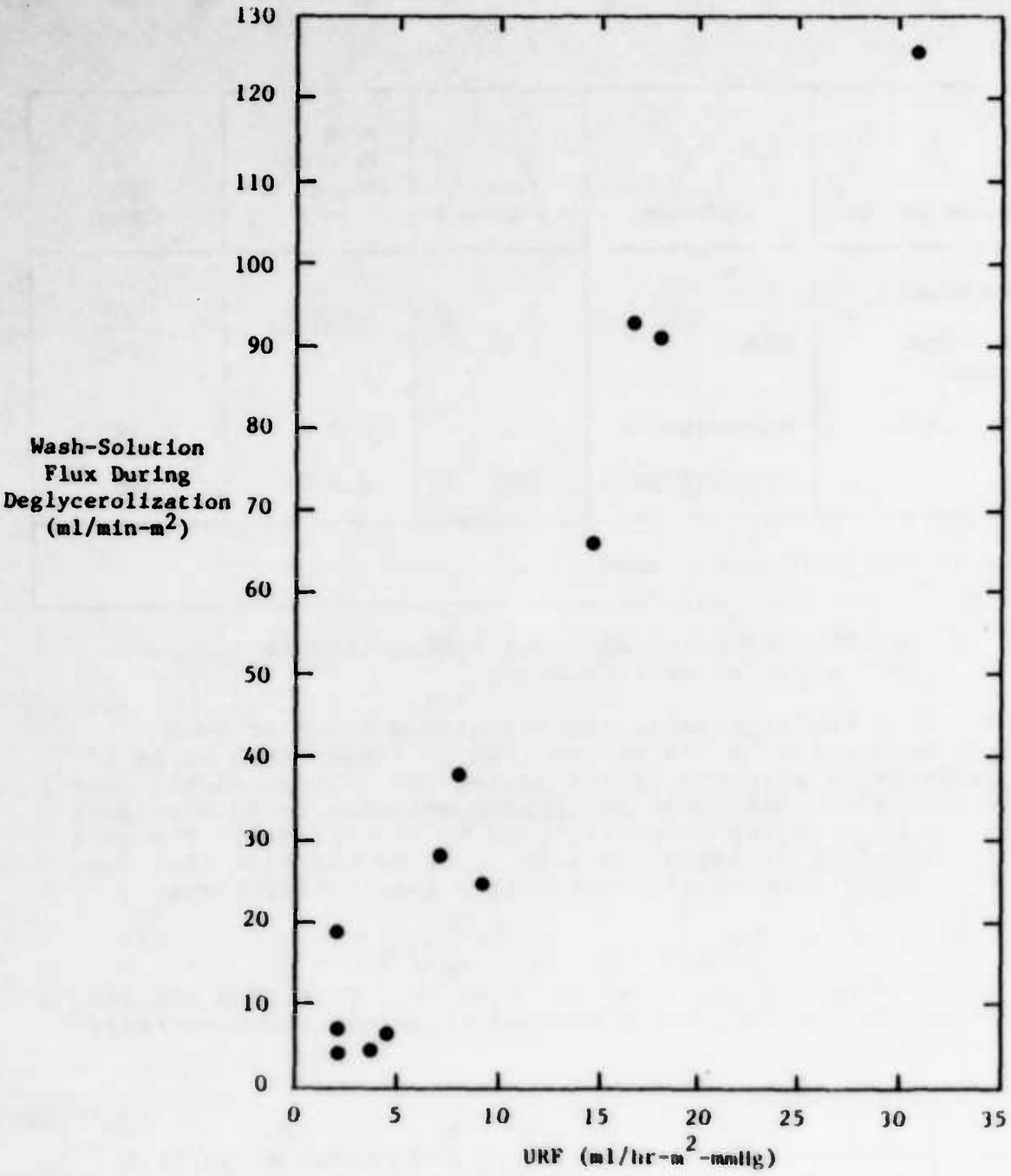


Figure 4. Correlation of Wash-Solution Flux During Deglycerolization with URF for Dialysis Membrane Devices

Legend: This figure is a plot of our experimental flux of wash solution during deglycerolization as a function of an ultrafiltration rate factor (URF) reported by the manufacturer. The URF is the ultrafiltration rate per unit TMP per unit membrane area. Each datum point represents the value for a different hemodialyzer or hemofilter.

concentration while the packed-RBC solution was being pumped through the test loop. The packed-RBC solution had been glycerolized and diluted with 150 ml of 12-wt% NaCl solution, then with 500 ml of saline wash solution according to the procedures given in Section III. The results of two iterations of this experiment are shown in Figure 5; they indicate that hemolysis was caused primarily by pumping the blood through the test loop rather than by processing it through the membrane device. The conclusion, then, is that the membrane devices do not contribute significantly to the hemolysis.

We then conducted parametric studies to determine the causes of hemolysis in our test loop. The parametric studies consisted of measuring the change in Hb concentration while processing the packed-RBC solution in different portions of the test loop (see Figure 2).

The packed-RBC solution in the blood reservoir is mixed by a stir bar within the reservoir or by an orbital mixer that shakes the entire reservoir. The changes in the plasma Hb concentration as a function of time with mixing by both methods are shown in Figure 6. It is obvious from comparing the increases in Hb concentration in Figure 6 with those in Figure 5 that the blood flow through the test loop rather than mixing in the blood reservoir is the major cause of hemolysis.

The packed-RBC solution is pumped through the test loop by a roller pump. We conducted experiments at constant blood-flow rate (230 ml/min) through a given length of tubing. In one experiment, the blood was pumped through the tubing by the roller pump; in the other experiment, gravity flow was used. The change in Hb concentration with time is shown in Figure 7 for these experiments. Based on these results, we concluded that the roller pump rather than the flow of blood is a major cause of hemolysis in our test loop during the deglycerolization experiments. In the future, we will use a peristaltic blood pump to minimize hemolysis.

In summary, membrane devices can effectively deglycerolize packed RBC. A few of the membrane devices exhibited deglycerolization performance that matched the performance of centrifuges. These results are impressive because the membranes were operated at the conditions recommended by the manufacturer for other uses--conditions that were not selected specifically for deglycerolizing packed RBCs. Optimization of the operating conditions for deglycerolization will improve the membrane devices' performance.

Task 2: Optimization Studies

The purpose of these studies is to optimize the operating conditions (TMP, blood-flow rate, wash-solution addition rate) for the deglycerolization procedure. Based on the results of our

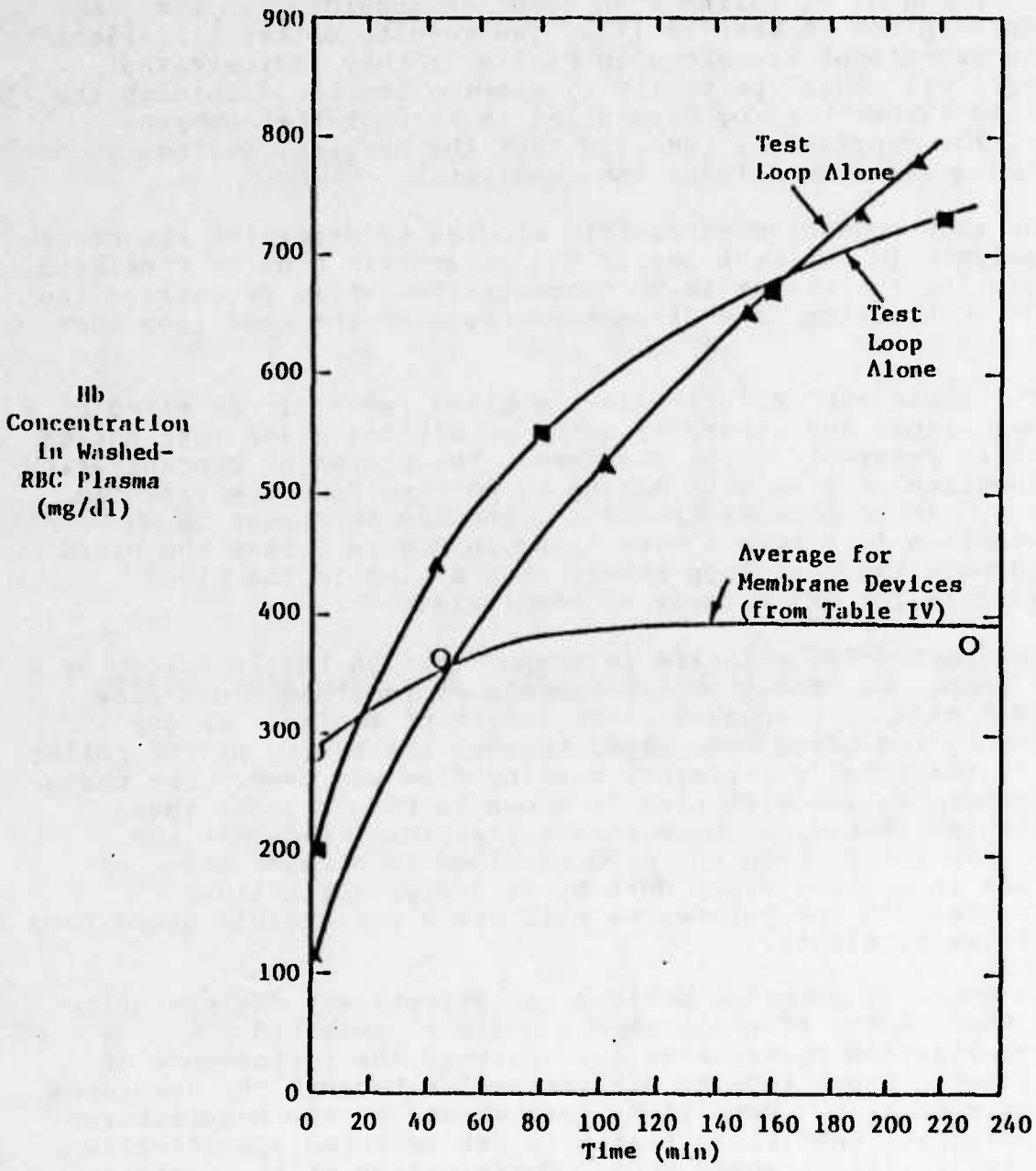


Figure 5. Hemolysis Test Results

Legend: This is a plot of the time-dependent change in the plasma Hb concentration in washed RBCs during deglycerolization in membrane devices and during circulation of blood through a test loop that did not contain a membrane device.

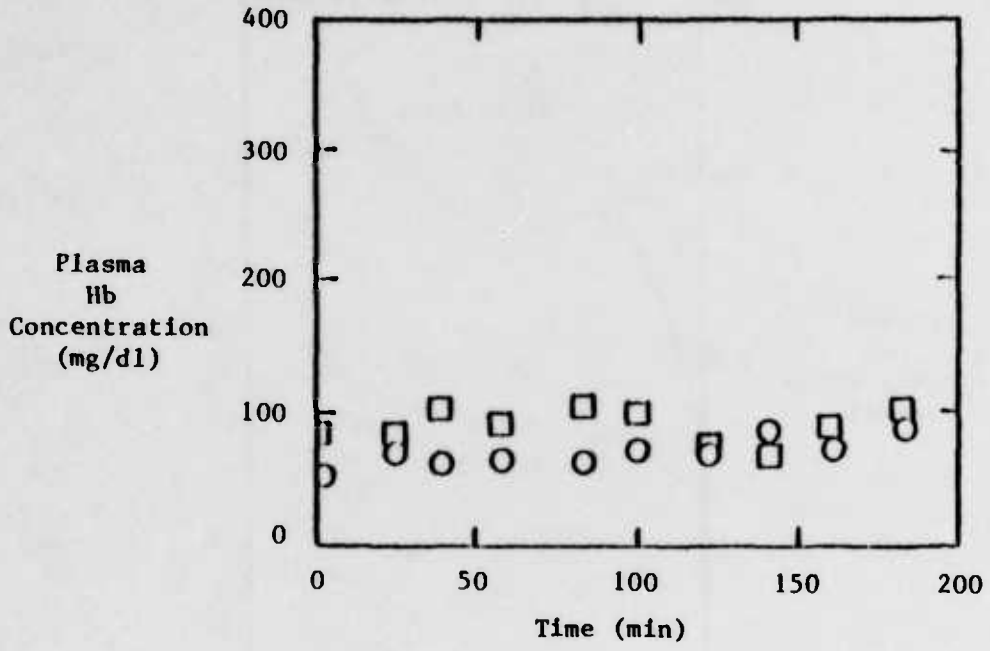


Figure 6. Effect of Stirring and Mixing on Hemolysis Without Blood Flow

o = shaker platform first half, stir bar second half
□ = stir bar first half, shaker platform second half

Legend: This is a plot of the time-dependent change of the plasma Hb concentration in blood when a magnetic stir bar or a shaker platform was used to mix blood in the reservoir. In one experiment (circular symbols), the mixing was done by the shaker platform for the first half of the time, then by the stir bar. In the second experiment (square symbols) the mixing was done by the stir bar first, then by the shaker platform.

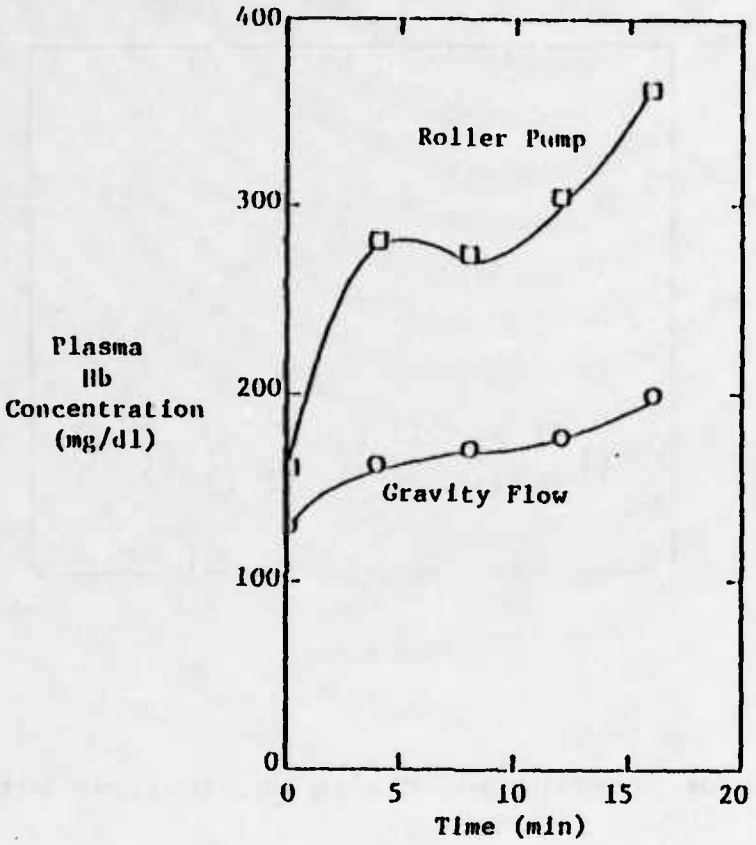


Figure 7. Effect of the Roller Pump on Hemolysis

Legend: This is a plot of the time-dependent change in the plasma Hb concentration in blood that is flowing at a rate of 230 ml/min through a given length of tubing. One curve (square symbols) shows the change in Hb concentration when the blood is pumped by the roller pump through the tubing. The other curve (circular symbols) shows the change in Hb concentration when the blood flows by gravity through the tubing.

feasibility studies, we selected seven membrane devices (Toray B1-200 and B1-L, Hemoflow F40, Biospal 1200S, Amicon D-30, Plasmapur, and Asahi Plasmaseparator) for the optimization studies. We conducted optimization studies with respect to TMP and blood-flow rate during the first year of the project.

A. TMP Studies

The purpose of these studies was to determine the TMP that resulted in the highest flux during deglycerolization. The average solution flux over the entire deglycerolization procedure as a function of the inlet TMP (at constant blood-flow rate) for each membrane device is presented in Figure 8. As expected, the flux increased for each device as the TMP increased, because the driving force for wash-solution transport across the membrane increased. The fluxes of the Plasmapur and Asahi Plasmaseparator microporous-membrane devices that are used for plasmapheresis were much higher than those of the devices that are used for hemodialysis or hemofiltration. The B1-L, B1-200, and the Biospal 1200S hemodialyzers were eliminated from further consideration because of these relatively low fluxes. We also eliminated the Amicon D-30 hemofilter from further testing. Although it exhibited about the same flux as that of the Hemoflow F40, the blood-side inlet of the Amicon D-30 plugged after processing one or two units of packed RBCs.

Evaluation of the deglycerolization performance of the membrane devices must also consider data on the change in the glycerol concentration in the RBC solution as a function of time and permeate (wash) volume. Recall that the membrane-based deglycerolization process should reduce the glycerol concentration in the RBC solution to less than 400 mOsm/kg $H_2O$ using about 2000 ml of wash solution in 35 to 45 minutes. Therefore, we measured the glycerol concentration in the washed RBCs as a function of time.

The decrease in glycerol concentration in the RBC solution as a function of time and permeate volume is shown in Figures 9 and 10 for the Hemoflow F40 hemodialyzer. Similar results were obtained for the other membranes in the TMP studies (see also Table VI). As shown in Figure 9, the Hemoflow F40 is capable of lowering the glycerol concetration to less than 400 mOsm/kg $H_2O$ in less than 30 minutes at TMPs of 450 mmHg and 325 mmHg, and in less than 45 minutes at 190 mmHg. However, the deglycerolization times are the same at 450 mmHg and 325 mmHg, even though the flux at 450 mmHg is significantly higher than the flux at 325 mmHg. If the membrane was limiting the speed of the deglycerolization process, a higher flux would result in a shorter deglycerolization time. Something besides the membrane is limiting the speed of the deglycerolization process; it is not yet known what that is.

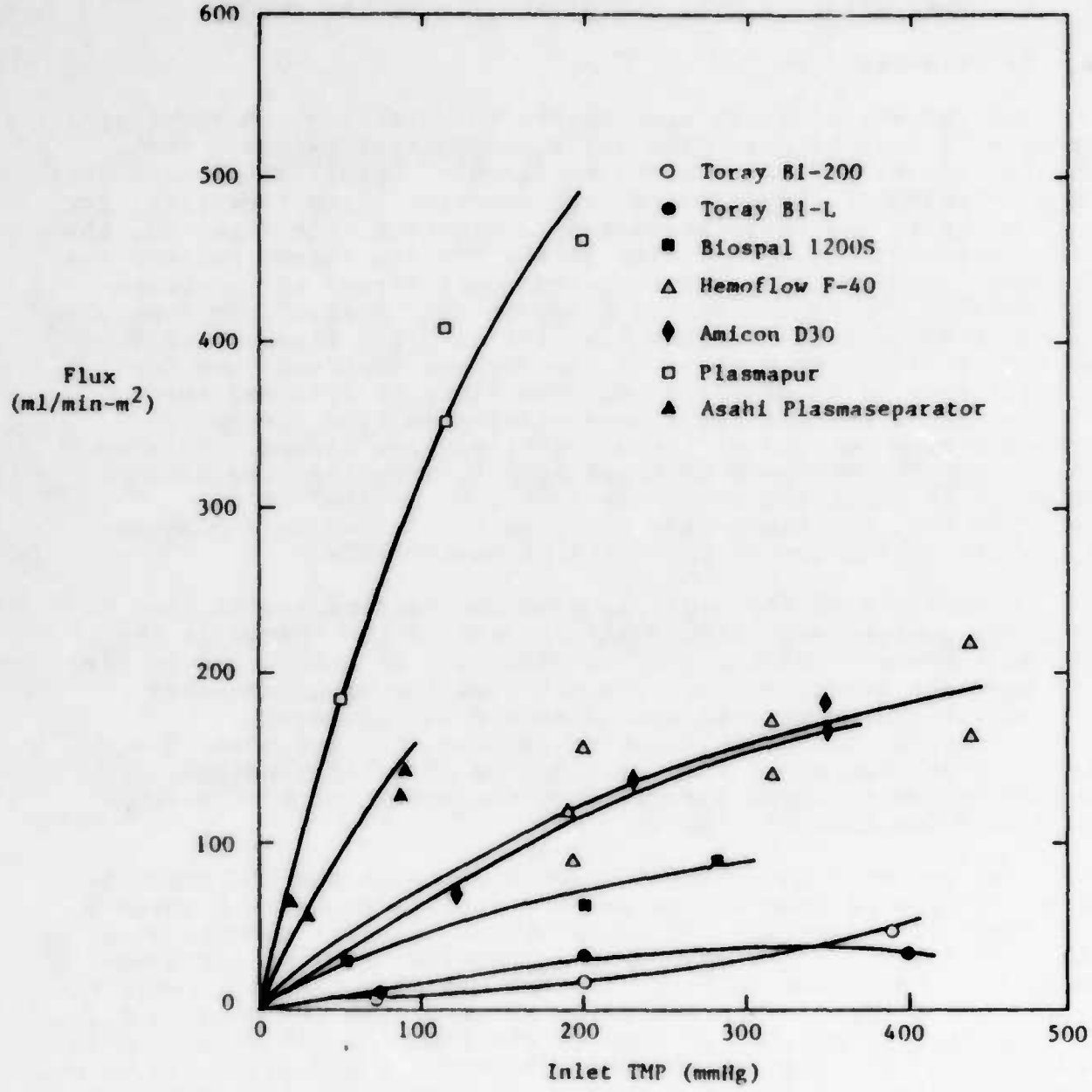


Figure 8. Effect of Inlet TMP on Flux During Deglycerolization

Legend: This is a plot of the average flux during deglycerolization as a function of the inlet TMP for seven different membrane devices. Each datum point represents the average flux for that membrane device during deglycerolization of a unit of packed RBC at a given TMP and constant blood-flow rate.

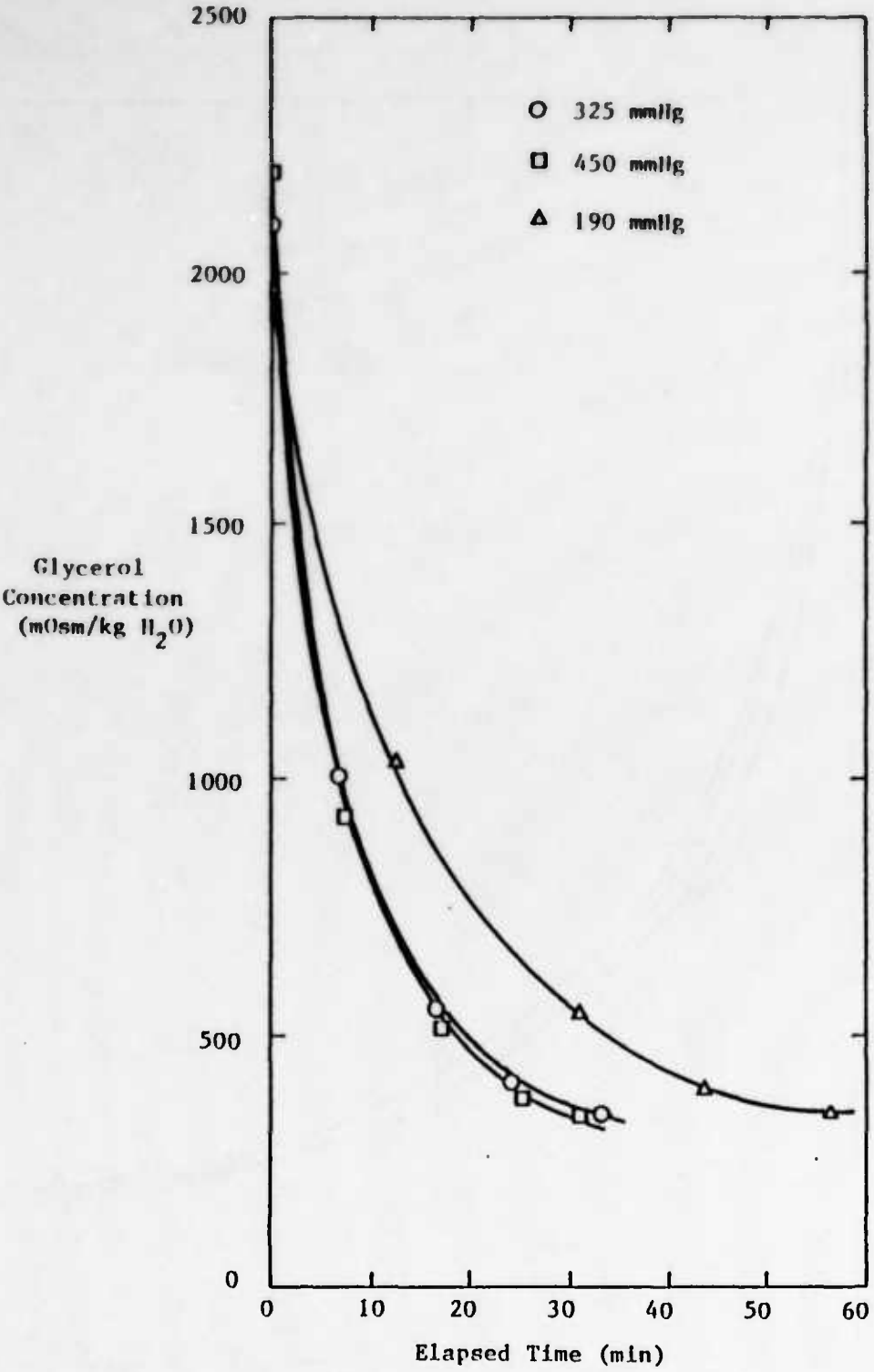


Figure 9. Effect of TMP on Glycerol-Removal Rate by the Hemoflow F40 Device

Legend: This figure shows the time-dependent glycerol concentration in the RBC solution at three different TMP for the Hemoflow F40 hemodialyzer. The blood-flow rate was constant at 230 ml/min. A single unit of blood was deglycerolized during the experiment.

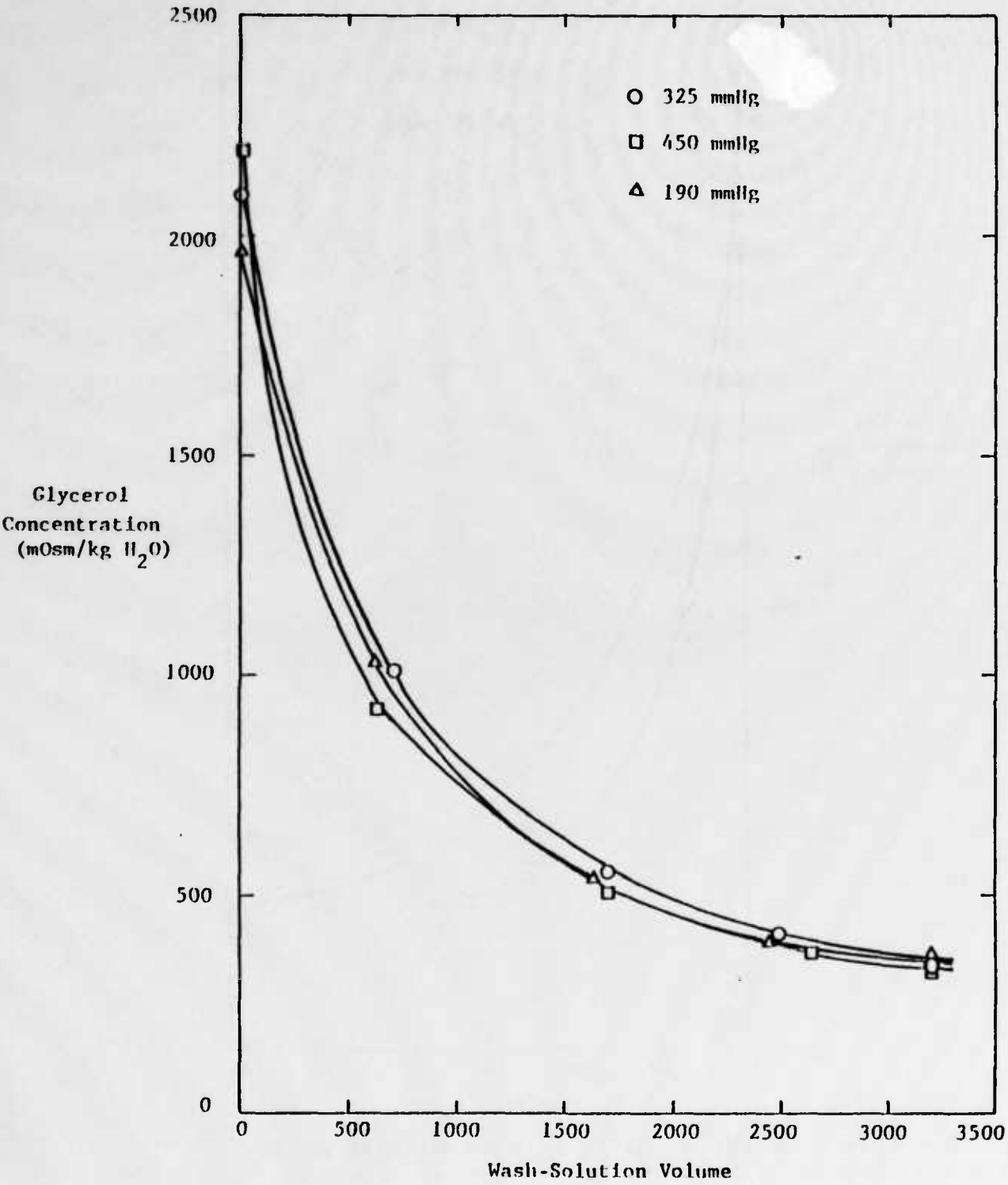


Figure 10. Decrease in Glycerol Concentration as a Function of Wash-Solution Volume at Different TMP for Hemoflow F40 Device

Legend: This figure shows the dependence of the glycerol concentration in the RBC solution on the wash-solution volume that is added during deglycerolization. The blood-flow rate was constant at 230 ml/min, but the TMP was varied. The device used was the Hemoflow F40 hemodialyzer.

| Membrane | TMP (mmHg) | Conditions at Which Glycerol Concentration in Permeate Was 400 mOsm/kg $H_2O$ | | | Final Plasma Hb Concentration (mg/dl) |
|---|---|---|---|---|---|
| | | Flux ($ml/min-m^2$) | Elapsed Time (min) | Permeate Volume (ml) | |
| Plasmapur* | 50 | 184 | 220 | 2800 | 293 |
| | 125 | 408 | 95 | 2300 | 60 |
| | 125 | 349 | 148 | 2300 | 415 |
| | 200 | 459 | 109 | 2620 | 140 |
| Hemoflow F40** | 190 | 120 | 30 | 2800 | 297 |
| | 190 | 82 | 45 | 2390 | 557 |
| | 200 | 159 | 26 | 2660 | 474 |
| | 325 | 172 | 19 | 2000 | 583 |
| | 325 | 138 | 25 | 2420 | 893 |
| | 450 | 205 | 15 | 1500 | 3933 |
| | 450 | 147 | 23 | 2220 | 820 |
| Asahi Plasma-separator+ | 15 | 66 | 14 | 1500 | 543 |
| | 30 | 50 | 24 | 2500 | 126 |
| | 90 | 127 | 30 | 2100 | 313 |
| | 90 | 138 | 40 | 2420 | 128 |

*, **Blood flow = 152 ml/min
+Blood flow = 230 ml/min
+Blood flow = 152 ml/min

Table VI. Effect of TMP on Membrane Performance During Deglycerolization

Legend: This table lists the result of our TMP studies using three membrane devices. The flux, elapsed time, and the cumulative permeate volume (equivalent to the wash-solution volume) when the glycerol in the permeate reached the standard of 400 mOsm/kg $H_2O$ are given as well as the concentration of Hb in the plasma of the deglycerolized packed RBC.

As shown in Figure 10, the wash-solution volume required to reach a glycerol concentration of less than 400 mOsm/kg $H_2O$ is greater than 2000 ml for all TMPs. This wash-solution volume is independent of the TMP and flux. These results also indicated that the deglycerolization process is limited by something besides the flux--perhaps the efficiency of mixing the blood and wash solution or the method of adding the wash solution. We believe the wash-solution volume can be reduced by changing the method of mixing or the procedure for adding wash solution. We will experiment with both as part of the later optimization studies.

The concentration of Hb in the plasma of the deglycerolized packed RBC is another important parameter that should be considered when evaluating the performance of the membrane devices. Recall that the plasma Hb concentration of the washed RBCs must be less than 150 mg/dl. We measured the plasma Hb in the deglycerolized RBC after each of the TMP experiments. As shown in Table VI, a few of the experiments met the standard. However, we could discern no relationship between the TMP and the final plasma Hb concentration. We suspect that this was due to allowing too many uncontrolled variables in the experiments, e.g., different types of blood, blood with different dates of expiration, and varying degrees of hemolysis in the test loop. All these variables would make different and unpredictable contributions to hemolysis. As mentioned previously, we will eliminate the roller pump as one cause for hemolysis by replacing it with a peristaltic pump that is specifically designed for pumping blood.

B. Studies of Blood-Flow Rate

The purpose of these studies is to determine the blood-flow rate that will result in the highest flux during removal of glycerol from the packed-RBC solution. The results of our initial optimization studies with respect to the blood-flow rate for the Plasmapur membrane device are shown in Figure 11 and Table VII. These tests were conducted at a constant inlet TMP of 125 mmHg. As shown in Figure 11, the average flux increased and the time to deglycerolize a unit of packed RBCs decreased as the blood-flow rate increased. The deglycerolization time could be easily decreased to less than 30 minutes by increasing the membrane area. (Recall that the Plasmapur membrane device contains only 0.07 $m^2$ membrane area.) According to the performance data provided by the manufacturer, the flux reaches a plateau as the blood-flow rate increases when the membrane is used for plasmapheresis. The reason this relationship did not hold true for deglycerolization is not known at this time.

As shown in Table VII, the volume of wash solution (which is equal to the permeate volume) required to deglycerolize the packed RBCs was not affected by the changes in blood-flow rate (and subsequent changes in the flux). This indicates that something besides the flux is limiting the speed of the

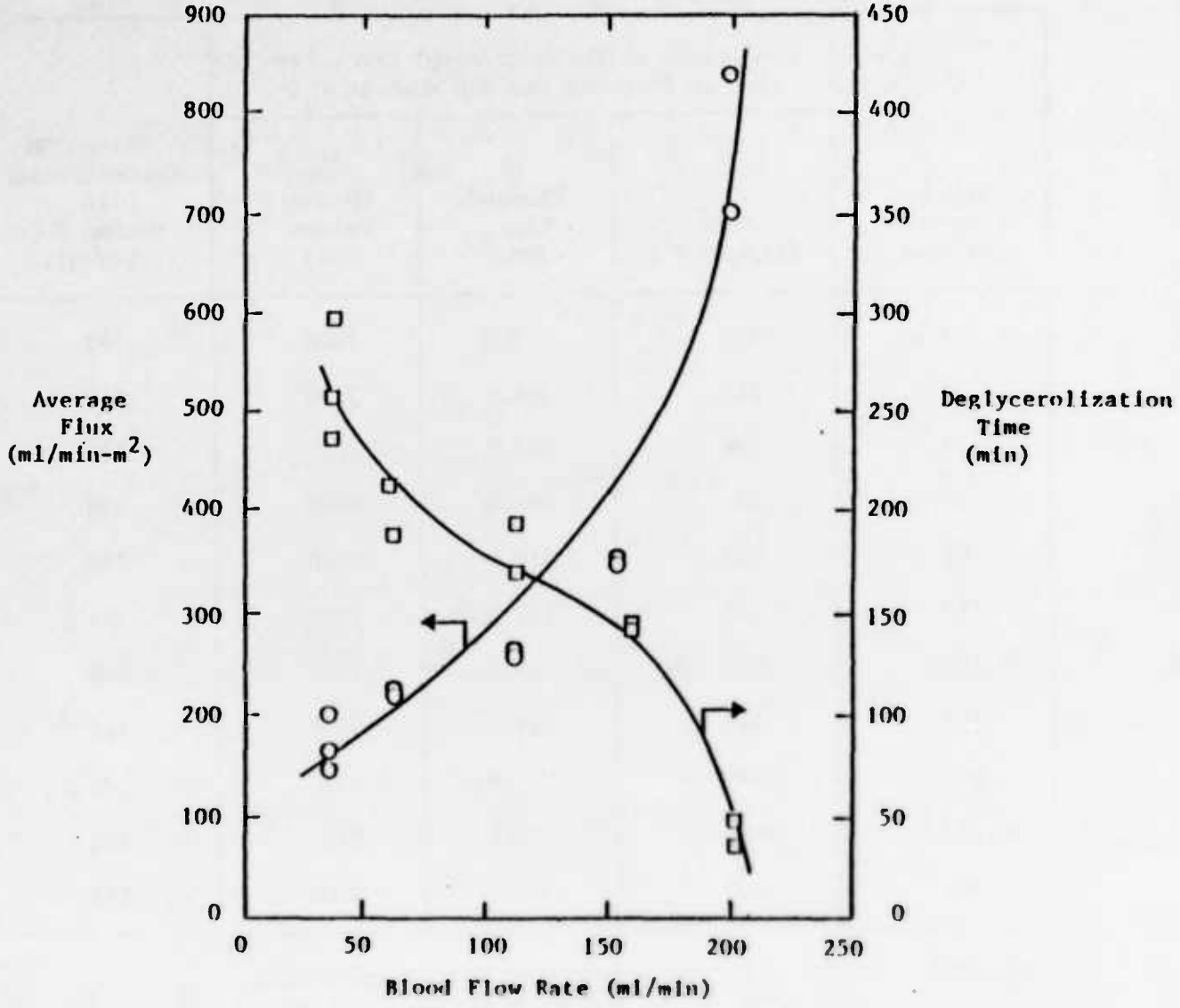


Figure 11. Flux and Deglycerolization Time as Function of Blood-Flow Rate at 125 mmHg TMP for Plasmapur Membrane Device

Legend: This is a plot of the dependence of the average flux (circular symbols) during deglycerolization on the blood-flow rate through a Plasmapur plasmapheresis device. The time that elapsed until the glycerol concentration in the permeate reached 400 mOsm/kg $H_2O$ is also plotted as a function of the blood-flow rate (square symbols). Each datum point represents the deglycerolization of a unit of packed RBCs at the given flow rate. The TMP was constant at 125 mmHg.

| Blood-Flow Rate (ml/min) | Conditions at Which Glycerol Concentration in Permeate Was 400 mOsm/kg $H_2O$ | | | Plasma Hb Concentration in Washed RBCs (mg/dl) |
|---|---|---|---|---|
| | Flux ($ml/min-m^2$) | Elapsed Time (min) | Permeate Volume (ml) | |
| 34 | 196 | 230.0 | 3120 | 181 |
| 34 | 165 | 258.0 | 3120 | 475 |
| 34 | 146 | 298.0 | 3120 | 170 |
| 62 | 219 | 184.0 | 2680 | 208 |
| 62 | 222 | 210.0 | 3080 | 193 |
| 110 | 251 | 172.0 | 2900 | 271 |
| 110 | 258 | 190.0 | 2820 | 260 |
| 152 | 345 | 149.0 | 3000 | 415 |
| 152 | 349 | 148.0 | 2900 | 370 |
| 200 | 840 | 37.5 | 2490 | 172 |
| 200 | 709 | 49.0 | 2600 | 146 |

Table VII. Effect of Blood-Flow Rate on Deglycerolization Performance of the Plasmapur Device (0.07 $m^2$) at TMP = 125 mmHg

Legend: The results of the optimization studies with respect to the blood-flow rate for the Plasmapur membrane device are listed in this table. The flux, the cumulative volume of permeate collected until the glycerol concentration in the permeate reached 400 mOsm/kg $H_2O$, and the time it took to reach this glycerol concentration are given. The plasma Hb concentration in the washed RBC is also given.

deglycerolization process. We suspect that the limiting factor is either the method of mixing the packed RBCs with the wash-solution, or the wash-solution addition procedure. The results of the earlier TMP studies (the required wash-solution volume was independent of TMP and flux) led us to the same conclusion.

As shown in Table VII, the final plasma Hb concentration at the highest blood-flow rate (200 ml/min) was less than 150 mg/dl for one test run, and less than 175 mg/dl for the other. Our earlier studies showed that blood flow through the test loop caused hemolysis rather than blood flow through the membrane device. Specifically, we suspect that the pump now being used in this project causes most of the hemolysis. We therefore believe that the target Hb concentration of 150 mg/dl can be met by the Plasmapur device if a peristaltic pump is used. This assumption will be checked in a test loop equipped with the type of peristaltic pump that is normally used for pumping blood.

During the next year, we will continue the flow-optimization studies at other TMP using the Plasmapur membrane device. The same series of flow-optimization studies conducted with the Plasmapur device will also be conducted with the Asahi Plasmaseparator and Hemoflow F40 membrane devices.

C. Factors Other Than the Membrane That Limit the Speed of the Deglycerolization Process

The results of the TMP studies and the blood-flow-rate studies indicated that other factors besides the membrane were controlling the rate of removal of glycerol from the packed RBCs. The reasons for this can be better understood by examining the deglycerolization system in more detail.

Up to this point, we have been optimizing the deglycerolization process by changing the operating conditions (TMP, blood-flow rate) only in the membrane device (see Figure 1). These changes directly affect the permeate-flow rate from the membrane. Increases in the permeate-flow rate will improve the efficiency of the deglycerolization process (i.e., will decrease the time and the amount of wash solution required to reduce the glycerol concentration in the blood reservoir) only if the membrane is the limiting factor. Since our experimental results did not confirm this, we know that other processes must be limiting the removal rate of glycerol, we believe these are processes that are occurring in the blood reservoir.

Consider the simplified diagram of the blood reservoir shown in Figure 12. For the sake of simplicity, only one RBC is drawn. Since the RBC has a higher concentration of glycerol than the reservoir solution has,[13] the glycerol moves out of the RBC and into the reservoir. This process occurs continuously because glycerol-rich solution is constantly being replaced by glycerol-lean solution and fresh wash solution. This results in a very low concentration of glycerol in the reservoir. The rate of

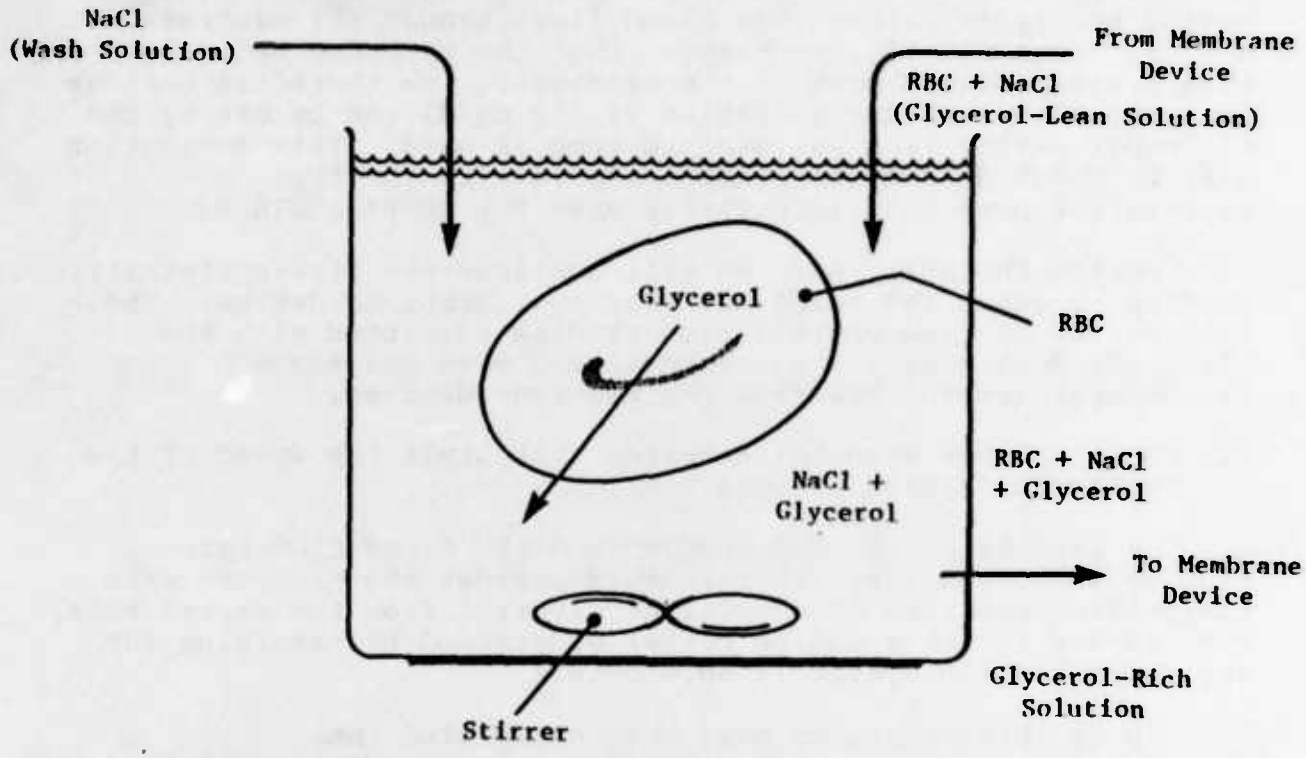


Figure 12. Simplified Diagram of Blood Reservoir

Legend: This is an idealized sketch of the blood reservoir. It shows a single RBC that contains glycerol. The wash solution in the reservoir extracts the glycerol from the RBC. The glycerol and some of the wash solution is removed by the membrane device while the RBC is returned to the reservoir. The addition of more wash solution removes more glycerol from the RBC.

addition of fresh wash solution and the mixing within the reservoir now determine how efficiently the glycerol is removed from the cell, because the membrane device does not limit how fast the glycerol is removed from the glycerol-rich solution.

The key to maintaining the fastest glycerol-removal rate from RBCs is to maintain the maximum concentration difference ($C_i - C_B$) between the inside of the RBC and the bulk of the reservoir (see Figure 13). This can be accomplished by changing the method of adding the wash solution, e.g., add a large volume of wash solution in a very short time, and increasing the efficiency of mixing the RBCs with the wash solution.

These types of experiments will be conducted during the second year of the contract as part of the optimization studies. Since the results of our studies during the first year have suggested that the methods of adding and mixing wash solution are limiting factors in the deglycerolization process, greater emphasis will be placed on studies to optimize those parameters. Consequently, the optimization studies will be extended for four months.

Task 3: Effectiveness Studies

The purpose of these studies is to determine the effectiveness of the membrane-based deglycerolization process at the optimum operating conditions using previously frozen, in-dated packed RBCs ("frozen" RBCs)--the type of blood the process will be operating on in actual use. All of our studies to date had used packed RBCs that had been equilibrated with a glycerol solution (see Section III for the experimental procedure). Units of packed RBCs are cheaper, easier to obtain, and easier to store than are frozen RBCs. These tests would indicate how good the glycerol-equilibrated packed RBCs were as models for frozen RBCs.

We used the three membrane devices that were selected for the optimization studies--Plasmapur, Asahi Plasmaseparator, and Hemoflow F40. The results of these tests are shown in Table VIII. The flux during deglycerolization of frozen RBCs was higher than the flux during the deglycerolization of packed RBCs. Thus, our results from studies that use packed RBCs are a conservative estimate of the expected performance using frozen RBCs. We will not use frozen RBCs again until we have completed the optimization studies in Task 2.

Task 4: Development of Reuse Procedures

The purpose of this task is to develop procedures for reusing the membrane devices. Although not a requirement, the ability of the membrane devices to deglycerolize more than one unit of blood would be beneficial for the military. We conducted preliminary tests to determine whether the membrane performance degraded as more units of blood were deglycerolized.

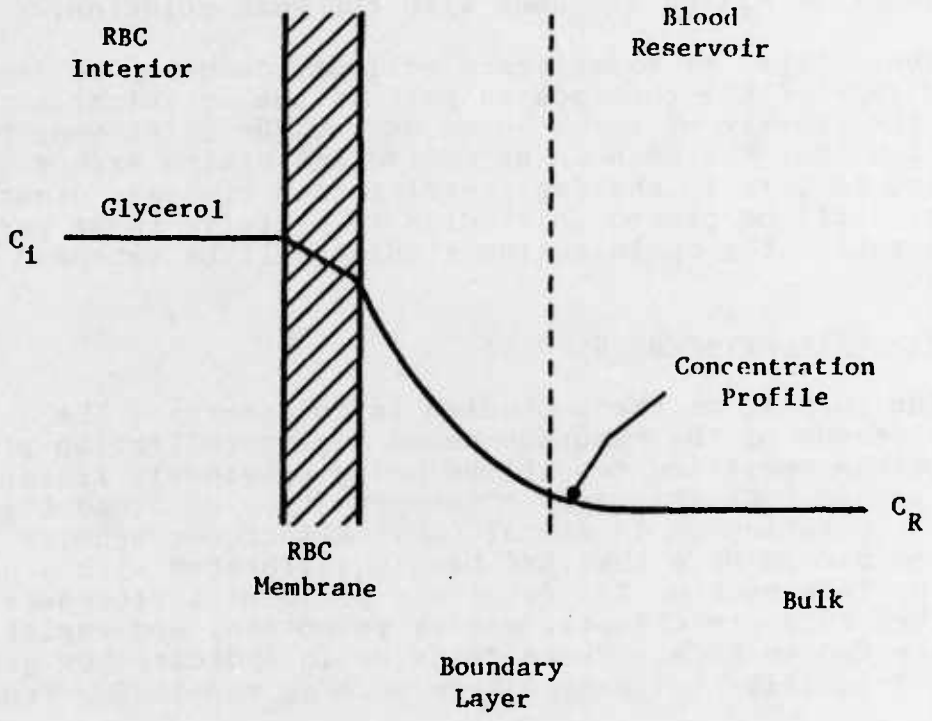


Figure 13. Simplified Cross Section of RBC and Blood Reservoir Showing the Glycerol-Concentration Profile

Legend: This figure is an idealized sketch of the concentration of glycerol inside the RBC and in the blood reservoir. It shows that the glycerol concentration in the cell, $C_i$, is higher than the concentration in the reservoir, $C_R$.

| Membrane Device | TMP (mmHg) | Blood Flow (ml/min) | Initial Glycerol Concentration (mOsm/kg $H_2O$) | Flux (ml/min-$m^2$) | Elapsed Time (min) | Permeate Volume (ml) |
|---|---|---|---|---|---|---|
| Frozen RBC | | | | | | |
| Plasmapur (0.07 $m^2$) | 200 | 152 | 2242 | 634 | 62 | 2600 |
| Asahi Plasmaseparator (0.5 $m^2$) | 90 | 152 | 2431 | 138 | 40 | 2400 |
| Hemoflow F40 (0.65 $m^2$) | 200 | 230 | 2179 | 159 | 26 | 2700 |
| Packed RBC | | | | | | |
| Plasmapur | 200 | 152 | 1996 | 459 | 109 | 2600 |
| Asahi Plasmaseparator | 90 | 152 | 2557 | 127 | 43 | 2700 |
| Hemoflow F40 | 190 | 230 | 1728 | 120 | 30 | 2800 |

Table VIII. Deglycerolization Performance of Selected Membrane Devices

Legend: This table lists the results of experiments that were designed to compare the deglycerolization of previously frozen RBCs with glycerol-equilibrated RBC. The TMP, blood-flow rate, and the initial glycerol concentration in the blood are listed for each test. Three membrane devices were used in these experiments. The flux, elapsed time and the cumulative permeate volume at the point when the concentration of glycerol in the permeate reached 400 mOsm/kg $H_2O$ (the maximum concentration allowed) are given in the table.

In these preliminary tests, the test loop and the membrane device were flushed with tap water, distilled water, and then normal saline solution after each deglycerolization procedure. The change in the average flux during deglycerolization of multiple units of packed RBCs in a Plasmapur device is shown in Figure 14. The flux decreased by about 25% after deglycerolizing five units of packed RBCs. As shown in Figure 14, the time required to remove the glycerol decreased slightly. Recall that the Plasmapur device contains only 0.07 $m^2$ membrane areas--about 10% of the area that would be used in a full-scale membrane system. Thus the processing time is relatively long using the smaller test device. Later in the project, we will resume deglycerolizing packed RBCs using the same membrane device until the flux decreases to about 50% of the initial value.

## VI. CONCLUSIONS

During the first year of this research contract to develop a membrane-based process for deglycerolizing previously frozen, packed RBCs, we successfully demonstrated that this could be accomplished with commercially available membrane devices. We completed all of Task 1: Feasibility Testing, and about half of Task 2: Optimization Studies. In addition, we conducted some preliminary work in Task 3: Effectiveness Studies, and in Task 4: Development of Reuse Procedures. Our work to date has demonstrated the following:

1. Commercially available hemodialyzers, hemofilters, and plasmapheresis devices can be used to effectively remove glycerol from packed RBC. Some of the devices removed the glycerol in less than 30 minutes--more rapidly than can a centrifuge.

2. The membrane devices removed the glycerol more efficiently from previously frozen RBCs than from glycerol-equilibrated, packed RBCs. This means that our studies with the packed RBCs give a conservative estimate of the expected performance of the membrane device.

3. As expected, the flux and removal rate can be increased by increasing the TMP and blood-flow rate in the membrane device. During the parametric studies, we showed that the rate of the deglycerolization process is not limited by the membrane. We suspect that the method of mixing the packed RBCs with the wash solution and the procedure for adding wash solution are the limiting factors. During the second year of the contract, we will direct some of our efforts toward improving the mixing method and changing the membrane-based deglycerolization process.

4. The roller pump in the test loop is a major cause of hemolysis during deglycerolization. Hemolysis is the reason the Hb concentration in the membrane-deglycerolized packed RBCs sometimes exceeded the 150 mg/dl standard. A peristaltic pump

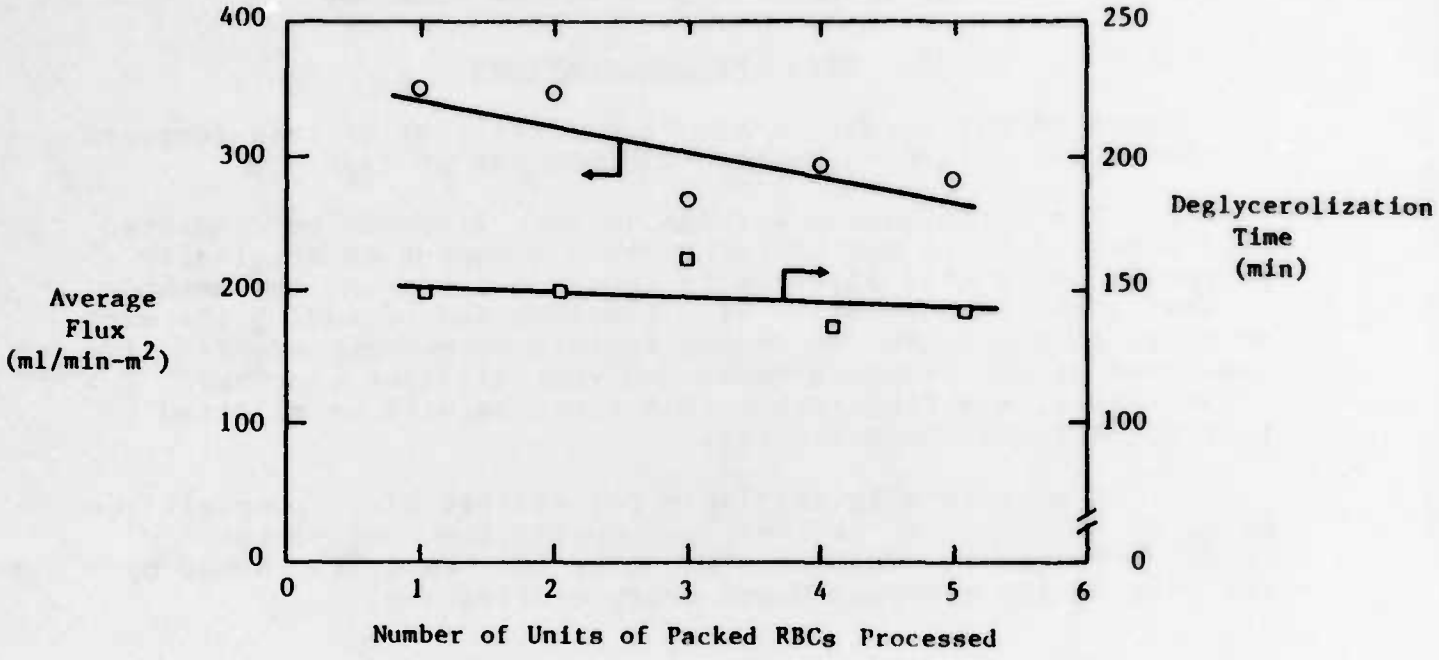


Figure 14. Flux and Deglycerolization Time as a Function of the Number of Fresh Units of Packed RBCs Deglycerolized in the Same Plasmapur Device

Legend: This figure shows the dependence of the average flux during deglycerolization on the number of units of packed RBCs that are deglycerolized in the same Plasmapur device. The change in the time it took to decrease the glycerol concentration in the permeate to 400 mOsm/kg $H_2O$ (the deglycerolization time) is also plotted as a function of the number of units of RBCs processed. The TMP was 125 mmHg and the blood-flow rate was 152 ml/min. After each unit of RBCs was deglycerolized, the Plasmapur device was flushed with tap water, distilled water, and then normal saline solution.

designed for use on blood will be used in future studies to minimize hemolysis and to confirm that hemolysis does not occur in the membrane device.

5. The studies performed in this program thus far indicate that a single membrane device can deglycerolize at least five units of packed RBCs without significant deterioration in its performance. We believe that a much greater number of units of packed RBCs can be processed without any problems. We will confirm this during the second year of the contract.

## VII. RECOMMENDATIONS

Based on our results during the first year of this research contract, the following recommendations can be made:

1. The optimization studies in Task 2 should be conducted over a period of 12 months rather than 8 months as originally proposed. This will allow us to focus on improving the methods of mixing the wash solution with the RBCs and of adding the wash solution to the packed RBCs--the factors we suspect are limiting the speed of the membrane-based deglycerolization process. Consequently, 4 months rather than 8 months will be allotted to Task 3: Effectiveness Studies.

2. A commercially available peristaltic blood pump will be obtained and used in the test loop during the rest of the research contract. This will minimize the hemolysis caused by the pump during membrane-based deglycerolization.

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